IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Examiner: S. Qazi

Application No.: 09/937,274

Group Art Unit 1616

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For: orally active 7.alpha.-alkyl androgens

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DECLARATION UNDER 37 C.F.R. 1.132

I, Marcel E. De Gooijer, declare as follows:

I am a pharmacologist, presently employed by N.V. Organon in the Netherlands as senior scientist in the pharmacology department.

I am, as member of a lead optimisation team, involved in pre-clinical research to find new androgenic compounds for medical use.

I am familiar with the contents of the patent application for which this declaration is submitted to the US Patent Office and I am familiar with the publication of Solo *et al*., Steroids, 40 (6), 1982, 603-614.

I declare that the information, which is provided in the following paragraphs of this declaration is a truthful description of results of experiments performed in the laboratories of Organon and filed as such in our archives.

In an in vitro assay androgenic activity of compounds was measured with Chinese hamster ovary (CHO) cells transfected with the human androgen receptor in combination with a mouse mammary tumor virus, and luciferase

receptor gene (incubation time 16 h, temperature 37 °C) and compared with the activity of 5α-dihydrotestosterone [according to the procedure described by Schoonen, W.G.E.J. *et al*, Analyt. Biochem. 261, 222-224 (1998)].

The $t_{1/2}$ of a compound after incubation with human hepatocytes was determined in hepatocytes collected from healthy young (25-45 year) male organ donors. The hepatocytes were cryo preserved in liquid nitrogen and kept there until use. These were thawed at 37 °C in a waterbath, placed immediately on ice, washed twice in one volume of cold (4 °C) incubation medium [William's medium E (without phenol red) with Glutamax I®, gentamicin 50 □g/ml, insulin 1 □M, hydrocortisone hemisuccinate 10 □M, fetal calf serum 0 % (v/v)], counted and the viability checked by Trypan blue exclusion. Cells were incubated as suspensions in 12-wells (non-coated) plates at a nominal density of 0.5 x $10^6$ cells/well in 1.5 ml medium at 37 °C with an air/$O_2$/$CO_2$ mixture (55/40/5). The plates were set on an orbital shaker at approximately 10 rpm.
The hepatocytes were incubated with 10 nM final concentration of the compound to be tested. The incubations were stopped after 0.5, 1 and 3 h by pipetting the whole incubation mixture into a glass tube and adding one volume of acetone on ice. The acetone was dried under a nitrogen flow at room temperature, the volume adjusted to 1.5 ml and the tubes were centrifuged at 4 °C at 10.000 x *g* for 30 min. The de-proteinized supernatants were collected for LC-MS/MS analysis.

The following results were obtained with these methods:
Table of results
**A**: Androgen receptor activity
**B**: Metabolic stability $t_{1/2}$ (min) with human hepatocytes

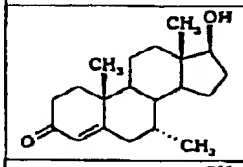
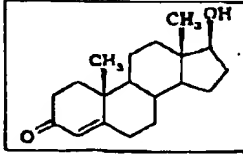
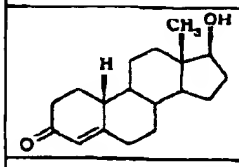
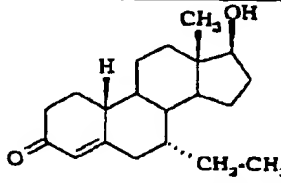
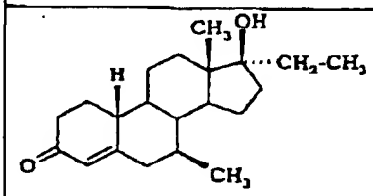
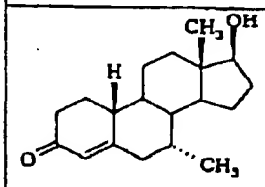
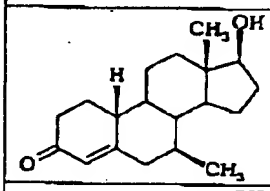
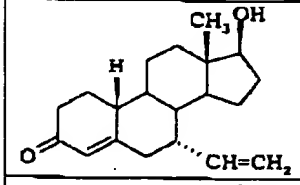
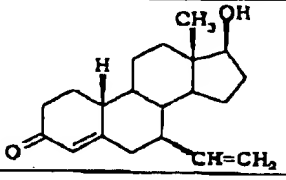
| Compound structure | Compound name | **Measurement results** | |
|---|---|---|---|
| | | **A** | **B** |
|  | 7α-methyl-testosterone | 45% | |
|  | testosterone | 16.5% | 15 min |

Table continued

| Compound structure | Compound name | Measurement results | |
|---|---|---|---|
| | | A | B |
|  | Nandrolone (19-nortestosterone | 55% | 16 min |
|  | 7α-ethyl-nandrolone (7α-ethyl, 17β-hydroxy estr-4-en-3-one) | 152% | 48 min |
|  | 7β-methyl-17α-ethyl nandrolone (17α-ethyl-17β-hydroxy-7β-methyl-estr-4-en-3-one) | 14% | |
|  | 7α-methyl nandrolone; MENT; 7α-methyl-19-nortestosterone | 269% | 20 min |
|  | 7β-methyl nandrolone | 14% | |
|  | 7α-vinyl nandrolone | 190% | 21 min |
|  | 7β-vinyl nandrolone | 8% | |

From these results I conclude that the comparison of MENT with 7β-methyl nandrolone or 7α-vinyl nandrolone with 7β-vinyl nandrolone, shows the major improvement in androgen receptor activation by selecting the 7α-stereoconfiguration.

Furthermore, although some activity is lost for the 7α-ethyl-nandrolone in the in vitro androgen receptor assay, there is higher activity by oral administration.

The results also show that testosterone analogues have much lower activity than nandrolone analogues. The superior activities of 7α-methyl and 7α-ethyl in the nandrolone series can not be derived from data obtained with testosterone analogues.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under 17 U.S.C. 1001 and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

Number of pages of this declaration: 4 pages.

2002- NOVEMBER - 26
Date

M. E. De Gooijer